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EXPERIMENTAL RESEARCH ON THE IMMUNOTHERAPY OF BURNS

Khirurgiya, No 9
Moscow, Sep 1955

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Profound many-phased functional and morphological changes occur in an organism as the result of burn trauma. On the basis of modern experimental and clinical data it is possible to establish schematically the succession of pathological phenomena which develop after a burn.

A stream of nerve and pain impulses from the site of action of the thermal irritant leads to disruption of the vitally important functions of blood circulation and respiration. Profound changes in metabolism, temperature regulation, and the composition of the blood occur. All these pathophysiological changes give rise to the clinical syndrome of burn shock. Neurodynic burn shock is the initial stage in a chain of subsequently developing pathological phenomena caused by severe burn trauma. The increase in the permeability of the capillaries with resulting loss of blood plasma from the blood stream into the interstitial spaces is a very critical pathogenetic factor. This brings about increased blood concentration, a decrease in the volume of circulating blood, and edema. In the process of the development of the burn syndrome, autointoxication is apparent even in its early stages. Massive destruction of cells and denaturation and toxic decomposition of proteins occur at the site of the burn. Toxic products are absorbed not only from the damaged tissues, but form as well in sites removed from the focus of direct injury. Sometimes lethal intoxication phenomena develop.

Autointoxication is an essential pathogenetic link in the development of the burn syndrome, a fact which is testified to by a large accumulation of experimental and clinical data. The existence of autointoxication is confirmed by cases in which experimental animals recover after radical excision of burned tissues. On the other hand, healthy animals, without exception, die after burned skin has been transplanted on them (M. P. Lebedeva). Experiments employing parabiosis showed that the parabiont which had not undergone injury died after experiencing exactly the same changes suffered by its burned partner (M. P. Lebedeva). It was found possible to save the lives of the experimental animals by administering substances which coagulate and adsorb proteins and the products of their decomposition.

Proceeding from what has been said above, the therapeutic tactics of the physician in treating patients with extensive burns consists of the following: (1) control of neuroreflex shock, (2) prevention or alleviation of severe autointoxication, (3) prophylaxis against and control of infection, and (4) creation of conditions favorable for the regeneration or plastic reconstruction of the skin.

Neurodynic shock and other early disorders can be alleviated or completely suppressed with the aid of therapy based on the principles of Pavlovian physiology, i.e., administration of morphine, the use of a novocain block according to A. V. Vishnevskiy, drug-induced sleep, the injection of E. A. Asratyan's liquid, etc. The transfusion of blood and plasma is also useful.

Thus, the control of neurodynic burn shock is completely possible at the present time with the aid of effective therapeutic measures. Therapeutic measures of comparable effectiveness still have not been proposed for the prevention or assuagement of fulminant autointoxication.

Autointoxication is an extreme trial for the organism of the patient and its compensatory physiological mechanisms. During this period in the development of the burn syndrome, profound functional changes occur in all the organs and physiological systems of the patients.

The results of our research are presented briefly in this article. Our work was conducted according to the following plan: (1) study of the toxic properties of the tissues and blood of burned dogs by biological methods, (2) study of the antigenic properties of blood and tissue proteins of burned animals with the aid of the method of anaphylaxis combined with desensitization, (3) the search for and study of methods of immunotherapy capable of preventing or alleviating autointoxication in the fatal form of burns in animals.

We conducted our experiments on dogs without the use of anesthesia. The burn was inflicted by a flame on a skin surface carefully cleared of hair. Two experimental types of burns were used: (1) burning of 20-25 percent of the total body surface with a 3-minute exposure, after which the animals which were not treated died within 3-5 days, and (2) burning of 38-42 percent of the total body surface with a 3-minute exposure, after which the untreated animals died within 6-36 hours. The burns were of third and fourth degree intensity.

Changes in the circulatory system as manifested by arterial and venous blood pressure, the results of radioactive measurements of the volume of circulating blood, roentgenokymographs, and electrocardiographs were studied in the experimental dogs during various phases in the development of the burn syndrome. In addition, studies were made of the morphological composition of the blood, the percentage of serum proteins, the cellular composition of the bone marrow, certain physicochemical characteristics of the protein systems of the blood, oxidation processes, and pathoanatomical changes.

Numerous experiments showed that toxic components with a complex biological action were formed constantly in the burned skin of the dogs. Introduction of 4 ml of a (1:3) aqueous salt solution extract taken from the skin of burned dogs within 24-30 hours into the blood of rabbits caused the latter to die quickly with attendant signs of muscular weakness, exhaustion, and involuntary defecation and urination. Upon dissection, large blood clots were found in the major blood vessels and heart. In control experiments, the internal infusion of an analogous extract from the skin of healthy dogs had no essential effect on the behavior of rabbits. The toxicity of burned skin is evidently connected with the presence of thrombin-like substances which cause rapid intravascular coagulation of the blood. It is interesting that similarly acting thrombin-like components were observed by Geyl'brunn [Hei'brunn] and his co-workers in the tissues of animals which had been subjected to general heating.

Our experiments showed that the direct toxic effect could be avoided with the aid of heparin. Study of the properties of the thrombin-like substance indicated that it was thermolabile and would not pass through a Seitz filter. The question then arises: is the thrombin-like substance the only toxically acting component of the salt solution extract obtained from burnt skin? It was later shown that the salt solution extract obtained from the skin, after having been freed of the thrombin-like substance by passing it through a Seitz filter, still exhibited its toxic action. Injection of this material caused rabbits to die within 3-8 days.

On the basis of our experiments it is evident that there are at least two toxically acting components in the salt solution extract taken from burnt skin. The first is thrombin-like, thermolabile, does not pass through a fine-pored filter, and has a fatal effect on the animal within a short period of time. The second passes through the filter and exerts its effect after a specific, more or less prolonged incubation period.

There are different opinions regarding the chemical nature of the toxigenic substances formed in burnt tissues. According to the data of some investigators, the toxic substances are formed by the splitting of the proteins of the burnt tissue, and also, possibly, in areas removed from the site where the burn stimulus is applied (Billroth, Davidson, I. N. Ishchenko, M. P. Lebedeva, and D. Ye. Ryvkina). In the opinion of other authors, the source of the toxic products is the blood which has been subjected to mortification due to the burn. V. A. Negovskiy presented proofs that toxic products are formed in tissues of the brain subsequently to burns. In contemporary literature there are indications that the burn syndrome is a manifestation of protein autointoxication (I. N. Ishchenko, M. P. Lebedeva, and F. Shutte). Our own research has revealed new evidence of the importance of proteins in the process of burn intoxication.

P. S. Vasil'yev and V. V. Suzdaleva conducted an investigation, in conjunction with us, on the protein systems of the blood of dogs before and after the infliction of burns. The protein content in the blood serum was determined. An analysis of the fractional composition of the protein with the aid of the fractional salting-out method was carried out. In addition, the lability of the protein systems was studied by evaluating the reactions of proteins in response to the denaturing effect of heat through the method of determining the gelatinization time and the resistance to alcohol. The experiments which were conducted showed that the infliction of a burn trauma led to severe quantitative and qualitative changes in the protein systems of the blood. Within the first minute after infliction of the burn, the protein content increases and changes occur in corresponding protein fractions; i.e., there is an increase in the albumin-globulin (A/G) coefficient. After 24 hours, the period of the development of acute intoxication, there is invariably a strong stabilization of protein, a fact which is manifested by a significant increase in the time required for the gelatinization of serum. The proteins lose their normal capacity to react in response to a denaturing thermal influence and the time required for the formation of a gel increases. Animals which are not treated die after exhibiting stabilization of their protein systems, a high protein content in the blood, and an increased A/G coefficient.

Such profound changes in the physicochemical structure of proteins after a burn, in our opinion, cannot fail to exert an effect on their specific biological characteristics. We undertook special investigations concerning the antigenic characteristics of the protein substances of burned tissues in experimental animals. In order to detect the autoantigenic protein substances we utilized the method of anaphylaxis accompanied by desensitization developed by L. A. Zil'ber to determine tumor autoantigens.

The experiments were conducted in the following manner. Guinea pigs were sensitized to a salt solution extract of the burned skin of dogs by subcutaneous injections. After 15-20 days they were desensitized by injecting an extract prepared from the skin of healthy dogs. After the animals had become areactive to the introduction of antigens from healthy skin they were again injected with an extract from burned skin in a dose not exceeding that which had been used to test for complete desensitization. The guinea pigs responded to this injection with anaphylactic shock of various degrees of severity.

With the aid of this method, we succeeded in the majority of cases in establishing the fact that specific antigens exist in the skin of burned animals. Specific burn autoantigens were detected in the blood serum and in a salt solution extract taken from the heart muscles of animals with burns. In order to resolve the problem as to whether the antigens which we had observed in the blood are identical with those present in the tissues of burned animals, guinea pigs were sensitized by salt solution extracts from burned skin, and then desensitized with extracts of normal skin and serum taken from the blood of healthy

dogs. After this, the internal injection of blood serum from burned dogs into the animals produced a symptom complex characteristic of anaphylactic shock in various degrees of severity. These experiments permit one to assume that the autoantigens which appear due to the effect of burns in the damaged tissue are transported into the general blood stream and into various tissues of the afflicted animals.

The establishment of the fact that specific antigens are present in burned tissues and also in the blood of experimental animals permitted us, in our efforts to resolve the problems inherent in the control of burn autointoxication, to channel into the right direction research on the development of methods of specific noninfectious immunotherapy for the burn syndrome. In setting up our research program we proceeded from the working hypothesis that the autoantigens were instruments of the toxic action. One can suppose from this that specific humoral defense factors are elaborated in an organism subjected to burns. Consequently, serum from humans or animals who have recovered from burns when injected parenterally into newly burned individuals should exhibit an antitoxic, i.e., therapeutic action.

In connection with this, one ought to remember the work of G. I. Segal' (1938) which showed that serum and blood of persons who had recovered from burns was used in isolated cases for the treatment of burned patients. Unfortunately, this work was not commented on in the literature. The latter fact is probably explained by the fact that the author did not give sufficient credit to the positive action of the serum taken from recovered patients, and his investigations did not go beyond the realm of empirical research.

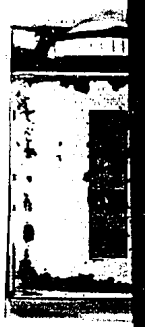
Research on the experimental immunotherapy of the burn syndrome served the following purposes: (1) a study of the therapeutic action of serum from recovered burn patients; (2) a study of the therapeutic action of isoimmune (antiburn) serum; (3) the study of serum obtained after immunization with heterogenous burn antigens.

To determine the degree of therapeutic effectiveness of immunotherapy as compared with other types of hemotherapy and protein therapy now used to treat burns, we conducted numerous control experiments in which the following therapeutic media were employed: native isoserum in conjunction with morphine, tecodin, and polyvitamins; isoblood; preparations of the dextran type, i.e., the polyglykin [sic] TSOLIPK (A. A. Bagdasarov, G. Ya. Rozenberg) or colloidal infusin (M. A. Lisitsin, N. A. Fedorov, and P. S. Vasil'yev); and exchange blood transfusions (O. S. Glozman and A. B. Kasatkina). All these therapeutic media were tested in experiments on 31 dogs subjected to burns inflicted by the second variation of the method described above.

In all cases, the enumerated types of therapy did not save the lives of the animals and did not prolong them essentially in comparison with the untreated animals (Table 1).

Let us return to our experiments on the study of the therapeutic effectiveness of serum from recovered burn cases. We obtained serum from dogs which had been subjected to sublethal burns. The burns were inflicted on 9-15 percent of the skin surface by exposure to a flame for a time amounting to one minute. In a number of experiments we resorted to repeated burns.

In the first series, experiments were carried out on dogs, 40-42 percent of whose total skin surface had been subjected to burning for three minutes. After such an injury, the control animals, as is evident from Table 1, died within 6-48 hours. Experiments were carried out on 59 dogs in this series. Serum was usually infused an hour after infliction of the burn trauma at the rate of 10-15 ml per kilogram of weight.



The positive effect of infusing serum from recovered burn cases was completely evident and left no doubts. Especially remarkable improvement in the condition of the animals was noticeable on the day following infusion of the serum: the animals regained consciousness, they reacted in a lively manner to environmental influences, they developed an appetite, and no symptoms of toxemia were observed in them. The control animals treated by the media enumerated above were in a critical state by this time: they were in a complete state of prostration and retched constantly, their pulses were intermittent and irregular, their respiration was shallow. In all our experiments where convalescent serum was used we were successful in bringing the animals out of the state of burn autointoxication.

Later, after 3-4 days, a process of denudation (peeling of the skin) began in the animals in this series, and suppuration appeared on the 5th or 6th day, sometimes accompanied by septicopyemic phenomena. In a number of cases, by the 10th-15th day denudation of the superficial and deep muscles had laid bare the ribs and the parietal pleura could be seen. The animals died of septicopyemia after various periods of time.

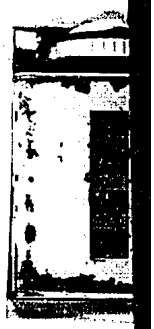
Because of conditions pertaining to our work, we did not succeed in preventing the sequelae of the burn, i.e., the generalized septic condition. In this series of experiments we only succeeded in making the animals resistant to burn intoxication and in prolonging their lives for as long as 21 days (Table 1).

In the second series of experiments we changed the method of producing the burns. The area of the burn remained the same as before, but the time of exposure was decreased to 1 1/2 minutes. The control animals in this series were treated with normal canine serum and lived 7-12 days. Under such experimental conditions, the therapeutic action of immunotransfusion was apparent to a still greater degree. Infusion of serum saved the dogs' lives. All the experimental animals survived (Table 2).

Experiments were set up to study the therapeutic effect of isoimmune serum and blood. To obtain therapeutic serum, healthy dogs were immunized by the internal infusion of the blood of dogs with severe burns. The immunization took place over the course of 3-4 weeks during which time 6-10 infusions of blood were administered. An increase in temperature, retching, and loss of appetite was observed in the animals after the first infusions. As time went on, they easily endured larger quantities of "burn" blood without any toxic manifestations. From 7 to 10 days after the last isoimmunization, blood was taken from the animals and therapeutic serum was obtained from it.

Transfusion of isoimmune serum to dogs with fatal burns caused a noticeable therapeutic effect: intoxication phenomena disappeared in the dogs, retching ceased, appetite appeared, and they reacted in a lively manner to their surroundings. Treatment with isoimmune serum of dogs suffering from burns of a type which killed the control dogs within 6-48 hours either prolonged their lives or saved their lives (Tables 1 and 2)

The positive effect of immunotherapy was established on the basis of a whole series of objective indicators. After the infusion of serum, arterial blood pressure rose a certain amount, pulse strength increased, respiration became deeper and less frequent, and the number of leukocytes diminished. Infusion of immune serum retards the development of burn oligemia, and also the tempo of development of the anemia which inevitably emerges at a later date after the infliction of a burn. A very clear therapeutic effect of immunotherapy is apparent from the study of the indexes of lability of the protein systems. In dogs treated with immune serum, as opposed to control animals, the lability of the protein systems is completely restored, and the



quantity of proteins returns to normal. The normalizing effect of immunotransfusion on the total quantity of organic acids in the blood can also be noticed. This testifies to the beneficial effect exerted on the metabolic oxidation processes in the presence of a burn syndrome (G. V. Derviz and V. N. Smidovich).

Recently we have produced evidence of the therapeutic action of convalescent serum and isoimmune serum in experiments on animals of another species. In addition we have succeeded in producing heteroimmune serum. To obtain this serum, we immunized goats and rabbits with an antigen isolated from the chemically injured skin of dogs. Experiments on immunotherapy employing heteroimmune serum were carried out on dogs using the methods indicated above. Intrasmuscular administration of alien serum also gave satisfactory results as manifested by a decrease in the symptoms of intoxication; i.e., the animals became lively, their temperatures went down, diuresis increased, and the tempo of development of oligemia was retarded. The length of life of such animals was essentially extended when compared with results obtained in control experiments in which the dogs were treated with nonimmune heterogenous serum (Table 2).

On the basis of the experimental research which has been conducted, one can draw conclusions concerning the high therapeutic effectiveness of methods on noninfectious immunotherapy in controlling burn intoxication. The therapeutic effectiveness of immunotherapy significantly exceeds that of the majority of methods of therapy proposed hitherto and studied by us in the past.

The results obtained are so convincing that we consider it possible to recommend that the pathogenetic method of immunotherapy for the burn syndrome be put into clinical practice. Recently, at our suggestion, extensive testing of the method of immunotherapy has been carried out on burn patients (D. M. Grozdov, S. A. Rovmov, L. G. Fishman, L. N. Pushkar', and V. P. Koshevaya).

On the basis of preliminary data, it is possible to state that immunotherapy is a highly effective method of treating the burn syndrome.

Table 1. Treatment With Immune Serum and Other Means of Dogs Suffering From Burns Covering 40-42 Percent of the Body Surface (3-Minute Exposure)

Type of Treatment	No of Experimental Animals	No of Days Animal Lived After Therapy				
		Less than one	1-5	6-10	11-15	16-21
Convalescent Serum	59	3	5	21	18	12
Isoimmune Serum	11	--	1	5	3	2
Heteroimmune Serum	7	--	1	4	2	--
Total	77	3	7	30	23	14

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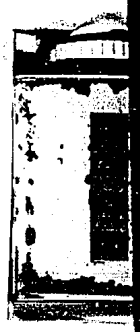

Type of Treatment	No of Experimental Animals	No of Days Animal Lived After Therapy: Less than one	1-5	6-10	11-15	16-21
		Control Experiments				
Isoimmune Serum and Isoplasma	10	7	3	--	--	--
Isoimmune Serum and Isoplasma in Conjuction With Anesthesia and Vitamins	5	2	3	--	--	--
Isoblood	4	4	--	--	--	--
Dextran	4	4	--	--	--	--
Exchange Transfusion	4	4	--	--	--	--
Colloidal Infusin	4	4	--	--	--	--
Total	31	25	6	--	--	--
Grand Total	108	28	13	30	23	14

Table 2. Treatment With Immune and Normal Serums of Dogs Suffering From Burns Covering 40-42 Percent of the Body Surface (Exposure for 1 1/2 Minutes)

Type of Treatment	No of Experimental Animals	No of Surviving Animals	No of Days Animal Lived After Therapy: 1-12	13-20	21-30	36
Convalescent Serum	7	7	--	--	--	--
Isoimmune Serum	6	5	--	--	1	--
Heteroimmune Serum	5	--	--	1	3	1
Total	18	12	--	1	4	1
		Control Experiments				
Normal Canine Serum	5	--	5	--	--	--
Normal Canine Serum	4	--	4	--	--	--
Normal Goat Serum	5	--	5	--	--	--
Total	14	--	14	--	--	--
Grand Total	32	12	14	1	4	1

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